Build Your Own Plant Tissue Culture Kit

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Grow and Propagate Plants Indoors from Seeds or Cuttings

Author's Name

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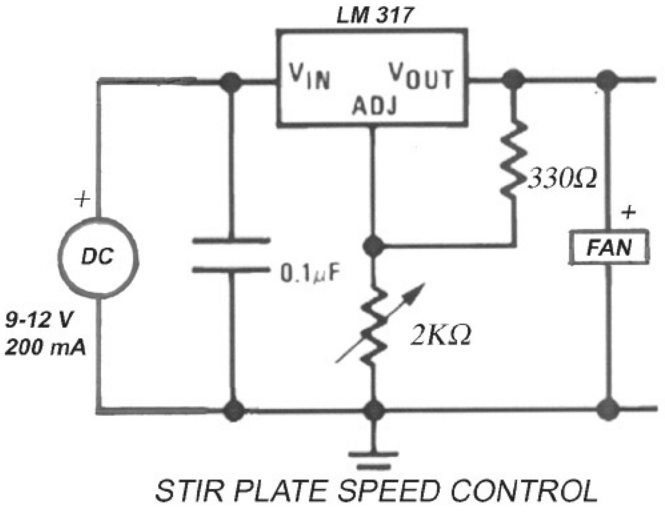
African Violet (Streptocarpus sect. Saintpaulia) is a common houseplant that can be propagated using tissue culture techniques.

Geraniums are a popular flower for putting on graves for Memorial Day.

Chrysanthemums come in many colors and styles.

Ornamental, edible, and insecticidal.



This circuit can be used to build a homemade magnetic stir plate using a computer cooling fan

Introduction

This book is the result of my own experimenting with growing plants from seeds or cuttings. I began experimenting with plant tissue culture as a way to grow and propagate plants during the winter months when it is too cold outside to garden. The idea was to be able to grow and propagate a variety of plants in a small space.

I had a lot of disappointing failures as I was trying to figure out how to get my plant cuttings and seeds cleaned up and growing in sterile conditions. Many of these failures were caused by not following the directions and trying to find an easier way of doing things. Besides using improvised, homemade media instead of the more expensive but proven commercial media, I also tended to experiment with the cleaning methods for the plant pieces and seeds. Often this resulted in either contaminated cultures or completely clean cultures where everything including the plant I wanted to grow was killed. The fear of contamination caused me to lose many plants that were left to dry up in their glass homes instead of taking them out to be divided and put into fresh media.

One of my dreams for this hobby was to make myself a portable culture collecting kit that would allow me to go out into the garden, field, or other places and take pieces of plants or seeds to grow myself. Supposedly, all that is needed is a small piece or even a single cell of a plant in order to use tissue culture techniques to make many more clones of the original plant. While this may be technically true, you still need to get it cleaned and into the right media with the right plant growth regulators. Especially with outdoor grown plants, there will be a lot of contamination needing removed or killed. Endophytic fungus and bacteria may also be a problem. You need enough plant materials to work with in order to account for some contaminated cultures and some killed ones. You need to get these plant pieces back to your lab without them drying out or turning to mush.

Also included in this book are tips, tricks, and interesting information I found online, in books, or from other people in emails web posts, forums, etc.

Photos are included where possible to illustrate the text, as examples, or simply to take up space. They are either my own pictures, public domain pictures found online, or other peoples pictures that were shared for use in this book.

I will likely go into way more detail than you want or need. This is done for my own records of my research and may or may not be entirely correct. Much of the information in this book is or can be dangerous. Even common, household chemicals can be dangerous and this book would never get written if I had to include all the warnings to keep everyone from getting hurt. If you are not willing and able to accept the responsibility for your own safety, don't try anything you find in this book. Do your own research and decide for yourself what you can and can't safely do.

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Keeping Notes

If you intend to experiment with tissue culture, seed starting, or propagating plants, you might want to keep good notes so you can repeat methods that have worked for you and troubleshoot any problems. For your own personal notebook, you won't likely have any rules you have to follow but you might want to adopt some ideas from laboratory notebooks. The exact requirements can vary a little but it should be a bound notebook and not a spiral bound or anything that has pages that can be torn out without leaving evidence. Graph paper pages might be helpful.

You should do all writing in ink so it can't be erased. Label or identify the notebook on the cover and first page or inside cover with your name, telephone number, address, project name or course number, or whatever seems relevant to you.

If your notebook doesn't have a prelabeled table of contents section, you should reserve several pages by writing "TABLE OF CONTENTS" on the top of those pages. You could use lower case Roman numerals for the table of contents pages and start regular Arabic numbers for the remaining pages. You don't need to number every page as soon as you start the notebook but staying a few pages ahead will help you avoid messing up or forgetting the numbering order. Number and date pages in a consistent manner. It could be the top right corner or the bottom center but stay consistent.

To avoid confusion, write out the month or abbreviation of the month instead of all in numbers. Jan 5, 2020 or 5 January '20 and not 1/5/2020.

If you choose not to use the backs of the pages, put a line from corner to corner to void all the spaces. You might use the backs for doing your calculations. In that case, void any remaining blank space. Don't erase anything or completely black out any mistakes. A single line ~~strikethrough~~ can be used to indicate a mistake but still leave it readable.

Your notes should be legible. Printing is a better idea than writing in cursive. You might be able to read and decipher your writing but it can make it hard for someone else to understand. There are exceptions and your writing might be very nice but generally printing is preferred.

The Table of Contents should contain the date the entry was made, the subject, and the page number the experiment starts on. This will allow you to quickly locate specific entries in the notebook.

Each entry is a detailed description of the experiments performed. It should include the date it was made, a title for the experiment, and a hypothesis or goal for the experiment. It should be complete enough that you could repeat the experiment at a later date or that someone else could do the same. It should list the protocols, calculations, ingredients, and equipment you used as well as any observations and results of the experiment. You can reference previous experiments, other published papers or other sources you used as guides or inspiration.

Be as detailed as possible. If the protocol called for 2 grams of something and you used 1.96 grams, that small difference might turn out to be important. If you notice a color change, the solution requires heating to dissolve, or a precipitate formed, write it down. Instead of just writing "Used 1 ml BAP solution" you could write "Used 1 ml BAP solution previously made on page 23" or "Used 1 ml stock solution of BAP (1 mg/ml) from the lab's stock". Besides giving enough details to repeat the experiment, it could help pinpoint troubles, problems, or different results between this experiment and previous or future experiments.

Problems and mistakes should also be noted. You can correct mistakes but don't remove them. Cross them out with a single line but leave it readable. Sign and date any corrections. Don't skip pages in a bound notebook and be sure to cross out any unused parts of a page. This prevents you or someone else from going back and adding things after the fact.

If an experiment will be continued at a future date and other experiments are entered in the meantime, you can note it something like "Cultures left to grow on shelf with 16/8 light/dark cycle … Continued on page ___". Don't leave space to continue later but making a note telling what page it continues on is a good addition.

Since this is likely your own personal notebook and not a university or company lab notebook, you can make it any way you like. This was just some possible ways to keep records of your experiments instead of a box full of scraps of paper.

Example:

Jan 29, 2021 Page 1

Title: Homemade Electrolytic Bleach Seed Surface Sterilization Protocol.

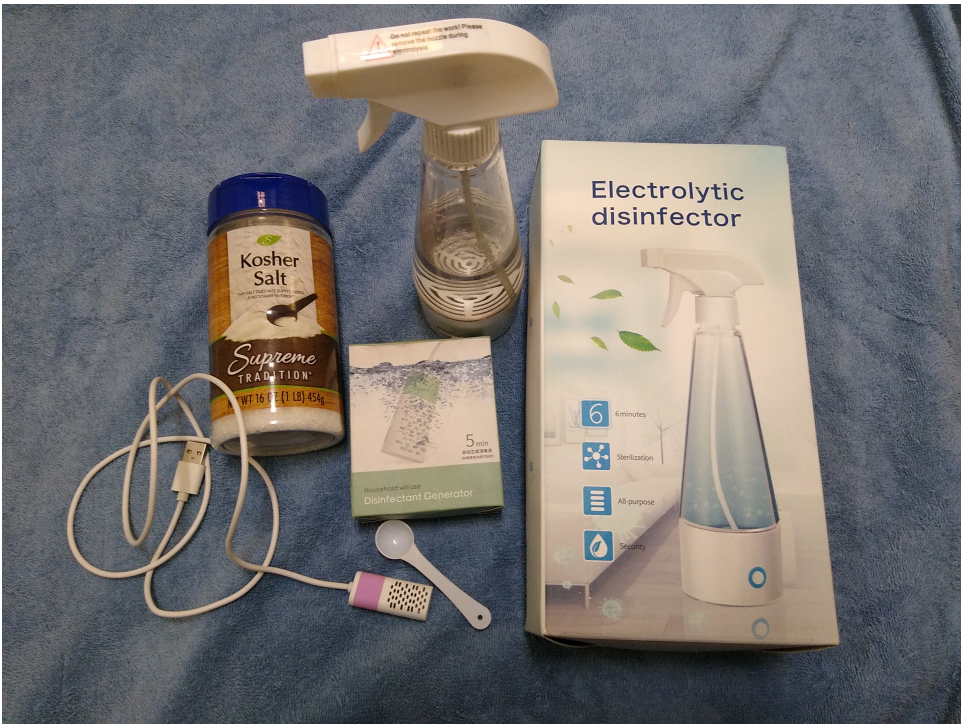
Purpose: To use a cheap disinfectant generator to produce the solution used for surface sterilizing radish seeds.

Introduction: A cheap disinfectant generator purchased online claims to make a sodium hypochlorite disinfectant using salt and water.

The device came with a measuring spoon that is supposed to hold 2 grams of table salt. The instructions say use 100 ml water and 1 spoonful of salt. The device is then put into the water and plugged into the power supply (5 Volts, 1 Amp USB adapter) and run for 10 minutes. The time can be increased for higher concentrations. The disinfectant produced could be stored in a sealed container for up to one week but I'll be using it immediately after making it to avoid any loss in strength.

The disinfectant produced will need diluted before using it but the concentration and time needed will be determined in this experiment.

Materials and Methods: USB Disinfectant Generator - I bought the cheapest device I could find on eBay. Normal non-iodized Kosher salt will be used but it will be weighed instead of using the included spoon. 100 milliliters of filtered spring water will be used. A small plastic jar will be used to hold the salt water as it is converted into the disinfectant. Top Line® brand Chlorine Test Strips were used to get an idea of the chlorine content made.



Since this is mainly a test of the disinfectant, a homemade tissue culture media was used instead of commercial media. Millipore 47 mm petri dishes were used because they are sterile, don't require a lot of media, and they are easy to examine for germination or contamination.

Radish sprouting seeds were used because they germinate fast and I have a big bag of them.

Procedures: Sodium hypochlorite concentrate is made.

1. The water was measured using a 50 ml centrifuge tube in place of a graduated cylinder. It was filled to the 50 ml mark 2 times and the water was dumped into a plastic container.
2. The salt was weighed out using a small digital scale to get 2 grams and mixed in the water until it was dissolved. The included spoon gave a little more than 2 grams using the Kosher salt.
3. The Disinfectant Generator was placed into the jar of salt water and plugged into the USB adapter.
4. A timer was started and, after 10 minutes, the generator was unplugged and removed.
5. The freshly made solution was put into a sealed glass bottle and will be used at various dilution rates for the testing.
6. A crude test for the chlorine content showed a 10% solution (5ml bleach to 45ml water) gave me between 25-50 ppm. A 20% solution (10ml bleach to 40ml water) gave me between 100-200 ppm. A 50% solution (25ml bleach to 25ml water) gave me between 400-500 ppm. A 75% solution (37.5ml bleach to 12.5ml water) Still showed between 400-500 ppm. Full strength it tested over 500 ppm (exceeded the range of the test strips).

This test was only done one time but it looks like at least a 20% solution would be needed as a "sterile" rinse water. At least 50%, 75%, or possibly even full strength would still be low enough that plants and seeds should handle at least a one hour soak but that will need testing.

A second test batch used 7.5 grams of salt in 100 ml water. Run for 10 minutes, a 10% solution gave 200-400 ppm and a 20% solution was at least 400-500 ppm.

The remaining 85 ml of the last bleach was given another 10 minutes using the generator. It showed around 500 ppm with a 10% solution. The 1 Amp power adapter got pretty warm during this 10 minute run.

One more test I went back to 2 grams of salt but ran the generator for 30 minutes.

Commercial bleach @ 5% available chlorine (rounded down) needs 1 ml in the 100 ml batch to get 500 ppm so it would be nice to produce this strength but even if it takes 10 times as much of the homemade bleach I'll be happy especially if it can be done using 2 grams salt per 100 ml.

When I used homemade bleach (not diluted) to spray the still air box and the outsides of plates, it left salt when it dried. Diluting it should lessen that problem.

OK, the 2 grams salt in 100 ml water gave about 500 ppm at a 10% solution. It is at the limit of the test strips but it should mean that a 20% solution would be around 1000 ppm. If I'm figuring right, full strength should be 5000 ppm or about a tenth the strength of commercial bleach but full strength should be similar to diluting commercial bleach to a 10% solution.

Another 30 minutes for the same batch and it looks like a 5% solution gives 500 ppm so the extra half hour seems to have doubled the strength. Full strength would now be 10,000 ppm or 1% chlorine.

I dumped some of the full strength bleach on a red shop rag. It lightened the color a little but nothing like real bleach right out of the bottle would.

Conclusion: Following the directions, 2 grams of salt (almost fill the included spoon) in 100 ml water and running it for 10 minutes is probably somewhere around 1000 ppm without any diluting.
2 grams of salt and 100 ml water running for 30 minutes gives about 500 ppm with a 10% dilution. Running it for a full hour gives about 1000 ppm with a 10% dilution.

Procedures: Media preparation.

1. Weigh out 2.5 grams table sugar, 0.05 grams water soluble 20-20-20 fertilizer, and 0.7 grams agar.
2. Mix dry ingredients with water to make 100 ml. in a small glass bottle and cover it with an aluminum foil cap.
3. Jars of water were prepared for use as sterile rinse water and placed in a pressure cooker along with a bottle of media.
4. Media and water were sterilized in the pressure cooker at 15 PSI for 20 minutes.
5. When the pressure cooker depressurized and cooled down a little, the still liquid media was removed, swirled to mix it up, and used to fill the petri dishes. A still air box was used to pour the petri dishes to help keep them clean.
6. The petri dishes and sterile water were allowed to cool before using them for the test.

Ideally, the petri dishes would be left in a clean space at room temperature for a few days or a week to make sure they are clean before using them but I wanted to get on with the experiment so didn't wait.

Procedures: Surface sterilizing seeds.

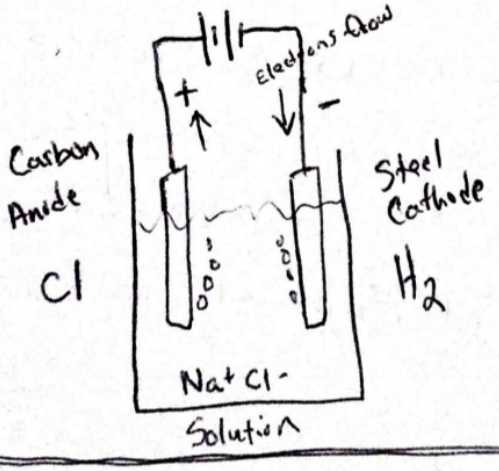
1. Sorted out 40-50 seeds to use in the test trying to avoid any that are damaged or don't look mature.
2. All of the seeds were put into a plastic jar with tap water and a drop of dish soap and washed for 10 minutes.
3. The seeds were rinsed 3 times in tap water for about a minute each time to remove the soap.
4. Seeds were placed on paper towels to avoid extra soaking time influencing the results.
5. Each test will use 25 ml of cleaning solution and two seeds will be washed in the solution for 15 minutes before being rinsed in sterile water and placed onto the media in the petri dishes.
6. Tests will use (parts bleach:parts water) - 1:0 (100% homemade bleach), 4:1 (80% homemade bleach), 1:1 (50% homemade bleach), 1:4 (20% homemade bleach), 1:9 (10% homemade bleach).
7. Tests will be repeated but soaking 30 minutes for each test.
8. Two control tests will be done using two seeds each soaked in tap water for 15 minutes and 30 minutes to make sure the seeds still germinate and that the soapy water and tap water rinse didn't kill or remove all the contamination.

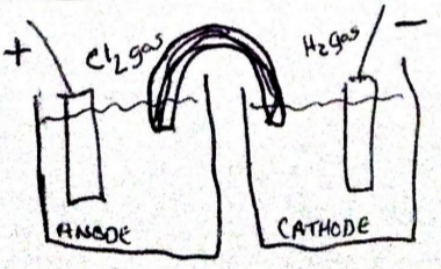
Continued on page _____

Electrolytic Production of Bleach

$$NaCl_{(aq.)} + H_2O = Cl_{2\,(g)} + H_{2\,(g)} + NaOH_{(aq.)}$$

$$Cl_{2\,(aq.)} + NaOH_{(aq.)} = NaClO_{(aq.)} + NaCl_{(aq.)} + H_2O$$





Example of a still air box with built in lights and hinged viewing window.

Still-Air Box

A still air box is just a place to do your tissue culture work in a cleaner environment. Protected from moving air currents, the still air has less chance of transferring contaminant containing dust particles into your sterile media. The box can be cleaned and sanitized to help ensure a clean workspace. Some people make a glove box instead of just a simple still air box. That is not really necessary. For one thing, attached gloves limit your movement and you won't have the same sense of touch. Another negative thing about a glove box is that it can create pressure or vacuum differences when moving the gloves around. This pressure difference can cause air to be drawn into the box through any cracks and possibly bring contaminants with it. They are more complicated to build than a simpler still air box.

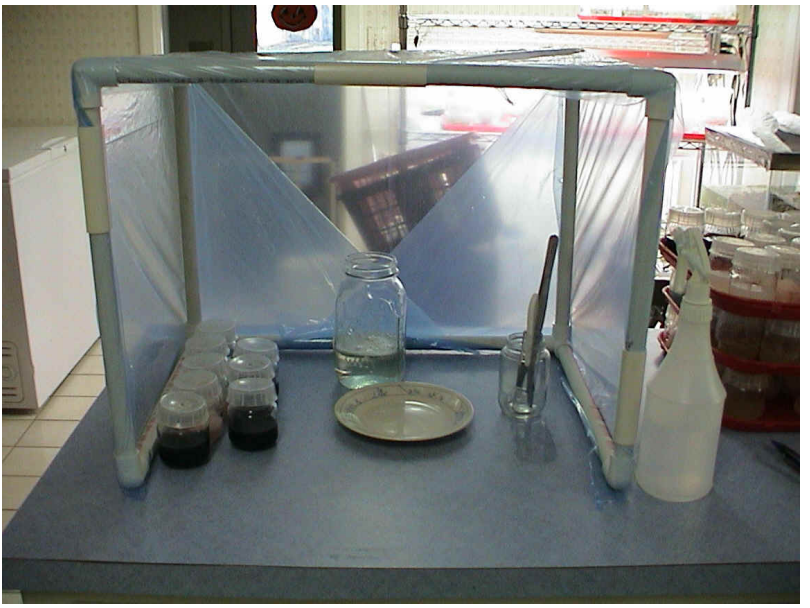
You can make your still air box as simple or as fancy as you like. A clear plastic storage box either turned on its side or with arm/hand holes cut in it is one common version. An aquarium turned on its side is another option.

The basic idea is just to provide a cover over the work so it blocks falling dust in the room from dropping into your work. In still air, dust won't move sideways. Any movements will stir up the air including the movements of your hands. You will need to pay attention and try to avoid unnecessary movements. Have your tools placed in a location where you can grab them without reaching over your sterile work. If you need to hold a tool in your left hand and that tool is on the right side of your box, hand it to yourself instead of reaching all the way across the box and over your clean workspace.

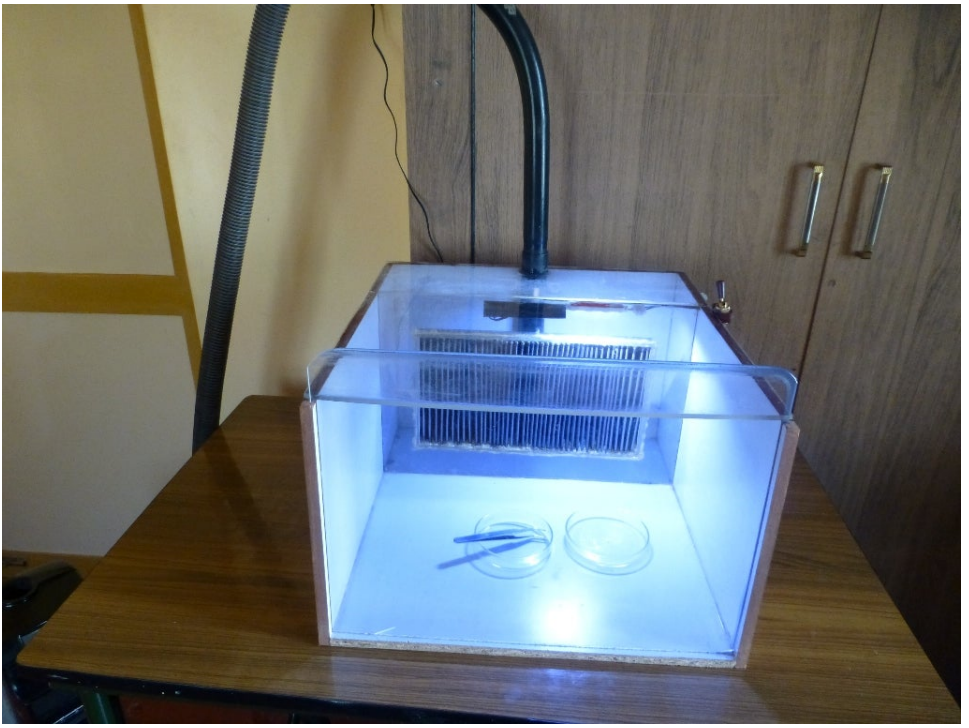
Take a large cardboard box and cut the top off. Cover the top with plastic or glass. Cut a hole or holes in the front the smallest size that will allow two arms to go into the box and work easily but no larger. Tape a flap

of clear plastic along the inside of the armholes and tape only at the top. This way as your arms go into the hole the flap will open. As you withdraw your hands the flap will close. Spray the inside of the box with Lysol. Put every piece of equipment that you will use into the glove box and spray it before you put it in. Put the Lysol can into the box. After everything is in, sterilize your hands and gently insert them into the armholes. Now spray your arms and hands and the inside of the box and everything in it with Lysol. Now everything is sterile you can go to work. Do not remove anything until you are done. The only opening into the box is a very tiny space where your arms enter the box. The probability of anything entering the box is so remote, that you may as well say it is sterile.

Cardboard isn't the best material to use but is good for testing out a design or as a quick improvised still air box.



This is another example of a still-air box made from a plastic pipe frame covered with plastic. It can be torn apart for storage or transporting.



Starting Simple

In some ways, plant tissue culture is like gardening on a miniature scale. Micropropagation or in vitro propagation is done inside the protected and controlled environment of a test tube, glass jar, or other container.

One of the first things you will need is the container which will house your plant. Test tubes or baby food jars are often used but might not be readily available to you.

You can just buy some test tubes online. 25mm diameter and 150mm long glass test tubes are a good size. Ideally, whatever vessel you choose as well as the lid or cover should be able to handle the temperatures of a pressure cooker or autoclave in order to sterilize them. Glass, polypropylene, polycarbonate, and metals are some examples of materials that can handle those temperatures. They might still soften and deform or break if they aren't vented to prevent pressure or vacuum.

For this first experiment, I'll keep things simple. Find yourself any clear container you have laying around. A glass or plastic jar will do. Even a drinking glass will work. Something similar in size to a baby food jar would be good but many things will do.

If your container has a lid, we'll use that. If not, aluminum foil or plastic will work. As I said, this will be a simple experiment so we won't be using a pressure cooker or other sterilizing methods. Simply wash your container out good to remove any of the previous contents. Stick with me here, there is a madness to my method.

Now you need your growing media. Since we aren't working with perfectly sterile conditions, we can't use the normal tissue culture media which contains sugar as well as agar to form a gel. Instead, we can use a soilless potting mixture, plain perlite, sand, or even cotton balls or balled up paper towels. Basically, we need something to support the seed or plant.

Potting soil can work but you might want to screen it to remove larger pieces. This will be more important if you use a test tube or other container that doesn't hold much media.

The sifted potting soil should still be loose but it will be free of sticks and other large particles. You want to mix in enough water to make it damp but not dripping wet.

Put an inch or so of the damp potting mixture into the test tube and pack it down loosely. A baby food jar or other container would be easier to fill but would take more media and more space. A small paint brush might help to clean up the sides of the test tube. A smaller tube or pipe (even a taped up paper tube) could be used to get the potting mix to the bottom of the tube while keeping the sides clean.

While it isn't completely necessary, it would be best to sterilize the growing media. This will help prevent anything from growing besides what you purposely put in. This contamination could be mold, fungus, algae, moss, other weeds, or even insects. To "sterilize" your media, first get your moisture level right. Add enough water to dampen the media but not so much that it turns into mud. Put it into a bowl or other microwave safe container and put it in the microwave until it is steaming hot. You only need enough media to cover the bottom of the container so this shouldn't take long.

Using a clean spoon, put your media into the container. If your container is microwave safe, you could have added the media first and microwaved it directly inside.

Cover the container with the lid, a piece of aluminum foil, or plastic. It won't need to be airtight. Just covered so dust and contaminants can't fall into it and to slow the evaporation so it won't dry out too fast.

You could make a plug for your test tube with a piece of cotton (synthetic fiber fill works good) wrapped in a cloth or paper towel.

You could put your seeds or cuttings in the media and call it done. There's a good chance the seeds would germinate or the cutting would root but part of this experiment is as practice cleaning or surface sterilizing the seeds or plant pieces.

The surface sterilizing process will differ depending on what you are cleaning. Some seeds or plant cuttings are pretty tough and can handle being washed and "scrubbed" with soapy water and a small paint brush. Other ones are delicate and will require much more careful cleaning.

For this example, I am going to use radish seeds. I bought a 1 pound bag of radish sprouting seeds because they grow fast but popcorn, beans, or any other seed you want to try should work. Since this is an experiment, I wouldn't suggest using any seeds you don't have enough of to sacrifice some in the process of finding out the strength and time needed to surface sterilize

them without killing them in the process. I also would choose something that doesn't take a long time to germinate and something you know can grow. It won't do any good if none of the seeds are any good to start with.

For larger seeds you might want multiple jars or a bigger jar but radish seeds are big enough to easily pick up with tweezers but small enough that I won't need a lot of space for this particular experiment.

I divided my jar into four sections and labeled each one with a time. In this case, 5 minutes, 10 minutes, 20 minutes, and 30 minutes. The very center will be used as a control with no treatment at all. I will put 2 seeds in each section removed from the solution at the chosen times to account for any bad seeds. My seeds are pretty clean to start with and the media doesn't have any added nutrients or sugar so you might not see any contamination growing even from the unwashed control. The goal here is to see at what point the cleaning solution kills the seeds. There is also the chance that none of the treatments times kills the seeds in which case you might want to repeat the test with a stronger strength cleaner or increase the time.

I am going to use regular household bleach for my test. A 10 percent bleach solution (1 part bleach to 9 parts water) is a common strength as is a 20 percent solution (1 part bleach to 4 parts water). In my case, I only need 10 seeds and they are not very big so I will mix up 25 ml of a 10 percent bleach solution by measuring out 2.5 ml of bleach and 22.5 ml of water. It just so happens that ½ US teaspoon is very close to 2.5 ml and 4-½ US teaspoons is almost 22.5 ml but it is the ratio and not the actual amounts that matters here so 1 part bleach and 9 parts water will be a 10 percent solution regardless of the total amount being made. Household bleach comes in different strengths but that won't matter as long as you are using the same strength with your next seeds.

I also want to rinse the seeds after soaking them to remove the stronger bleach solution from them before planting them. You could use boiled water for this or a very weak bleach solution. I'll use 5 drops of bleach in 25 ml of water which is probably stronger than really needed but should be weak enough not to cause problems with the seeds and is better than going straight from the stronger bleach solution into the media.

I pick out 10 nice looking seeds and set 2 of them aside as the control. Then I put all of the remaining 8 seeds in the bleach solution and start a timer. After 5 minutes, I remove 2 seeds, dip them in the weaker

bleach solution and plant them in the jar at the correct position. I try to place them right against the glass so I can see them from the outside. At each of the other chosen times I remove 2 seeds and repeat the process. After all of the soaked seeds have been planted, I take the control seeds and dip them in the rinsing solution before planting them in the center of the jar.

Now it is just a matter of waiting and watching to see at what point the seeds no longer grow. If they all grow, you could use a stronger bleach solution, leave them soaking for a longer time, or just figure the longest time should be good enough. When you start using a sterile media containing sugars and other nutrients you might need to do more tests if they are not clean enough.

With your sprouted seeds, you could transplant them to other pots or possibly directly into your garden or you can just dump them out.

The same experiment could be used with plant cuttings although you may need more than 25 ml of solution and possibly bigger jars. A cutting would probably benefit from a multi step cleaning process. Since cuttings will take longer than seeds to root and grow, I'll save that experiment for later but keep in mind that cleaning the cutting might increase the chances of success as long as it isn't damaged in the process. A longer rinse in sterile water may be helpful as well as trimming the ends and possibly the edges of the leaves to remove parts damaged from the bleach.

One possible plant you might want to propagate is a raspberry bush.

This is a raspberry branch from last season's growth. You can see the bud which is where the new growth will start.

With a normal cutting, you should have at least a couple of these buds so one or more would be below the surface as pints for roots to grow from. In this case, I'm only going to need one and hopefully the roots and new plant will form from the same point.

The branch is divided up leaving some extra to trim after the cleaning and surface sterilizing steps are done.

DIY Tissue Culture Media

In this experiment, I'll be making a DIY version of plant tissue culture media to use in place of the potting mix or other media we used in the previous experiments. Since this will be a more nutritious media, it is more likely to grow mold, bacteria, fungus, or other contamination so it is more important to sterilize everything and be careful not to introduce any contamination as you do the work.

The purpose of this experiment is to gain the confidence and experience you will use in later experiments. You are going to need more tools, equipment, chemicals, and specialized ingredients as you get deeper into this hobby but I will attempt to slowly walk you along as you learn the techniques and skills needed without spending a lot of money before you are sure you can do the work and that it is something you want to do.

I am not a professional and have never had any real training in tissue culture and I'm learning and experimenting as I write this book. I'll be giving you tips, tricks, and non-traditional secrets I have learned. Some of them may be considered dangerous. Some of them may be overly complicated and unnecessary. I will try to point out where extra caution is needed but keep in mind that everything can be dangerous and even if I tried to point out all the things that could cause trouble, I can't possibly anticipate them all so do this at your own risk.

For this DIY tissue culture media, I will keep things fairly simple. It will just use a water soluble fertilizer and sugar along with water. Agar could be used to form it into a gel but it might be harder to find locally.

Keeping this small scale, I'll be making a 100 ml batch which is not very much but no sense making a larger amount when there is a good chance you will make mistakes and your cultures will get contaminated.

I'm using a 20-20-20 water soluble fertilizer. I have been using 50 milligrams of this dry powdered fertilizer to make my 100 ml batches of media but you might not have a scale to weigh it. You should be able to mix the fertilizer up about half the strength called for on your package. In my case, about ¼ teaspoon to a quart (or liter) of water should work. Mix this up and use 100 ml of the liquid. I recommend you get some way to measure in metric but for this experiment a half cup will be close enough. You'll

want to be more accurate especially when using agar in the media but this one will not be super critical.

Now you need about 2 or 3 grams of table sugar. This is about ¼ to ½ teaspoon.

If you were going to use agar, you would need somewhere around 0.5 to 1 gram depending on the agar and how solid you want the media. About ⅛ teaspoon or a little more. This would be 1 Dash spoon or 2 Pinch spoons. For this experiment I will use a cotton ball in each jar or tube in place of the agar.

Normally you would check and adjust the pH of the media to between 5 and 6 (5.6-5.8) but, assuming you don't have a pH meter or pH papers, we aren't going to worry about it.

This media would either be poured into the jars or sterilized separately and then used to fill the sterile jars or tubes. Again, assuming you don't have a pressure cooker or autoclave to sterilize everything, I'll give you a different option.

To clean your jars or tubes, first wash them with soap and water then rinse them. Mix up a 10 percent solution of bleach with 1 part bleach to 9 parts water. Pour the bleach from one jar or tube into the next one making sure it contacts all the inside surfaces. It depends on what type of lids you are using if this can be used for them.

Leave the jars or tubes drain upside down a bit before you fill them.

Alternately, you could boil the jars and possibly lids like you would for canning jars. You could also carefully heat and then cool the glass jars in the oven for 45 minutes to an hour at 160°C (320°F) but this won't work if you are using plastic jars.

Next, add 1 drop (yes, just a single drop) of bleach to your liquid media and mix it in. Heat the media up just until it boils in the microwave. If you have used agar, you should heat it until the agar melts and mixes into the media. It can easily boil over so heat it a bit, stop and stir it, then heat some more.

Now, in a clean area, put a cotton ball in each jar and pour the hot media on it and in the jar. Try not to fully submerge the cotton ball. It will absorb the media but will be used to support the seed or plant out of the media. The heat as well as the drop of bleach will help sterilize the media, the cotton ball, and to some extent the jar. This isn't as fool proof as actually using a pressure cooker to sterilize everything but this is a low-budget, DIY method you can try.

Put the clean lids on or cover them with aluminum foil and let them cool.

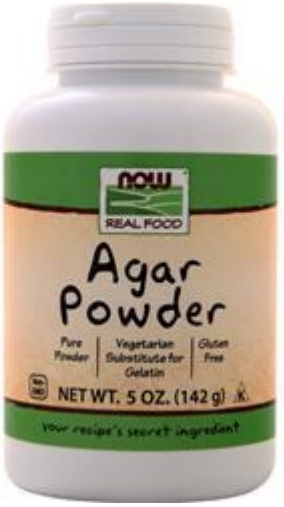
Use seeds or plant pieces cleaned like you did before in the jars on top of or stuck into the cotton balls. If everything goes right, the seeds will germinate or the plants will start to root and grow. If they get contaminated you will see fuzzy growth that isn't plant roots, the media might get cloudy, or you might see odd colors or other signs of contamination.

Agar and Substitutes

Agar is used to turn the nutrient media into a gel. It is a mix of carbohydrates that have been extracted from red algae, a type of seaweed. The seaweed is typically boiled into a gel, pressed, dried, and then crushed to form agar flakes, blended into a powder, freeze-dried into bars, or made into strands.

Agar is also known as agar-agar, Chinese gelatin, China grass, Chinese isinglass, Japanese gelatin, Japanese isinglass, kanten, vegetable gelatin, or vegetarian gelatin.

Telephone brand agar-agar is a common type used for tissue culture but there are other brands as well as bulk agar powder available in stores or online.



You might find it in a local health food store, Asian grocery store, or possibly in the baking or bulk foods section at your normal grocery store. You might have to order it online but shop around locally or online because prices can vary a lot. Make sure it is pure agar and not already flavored.

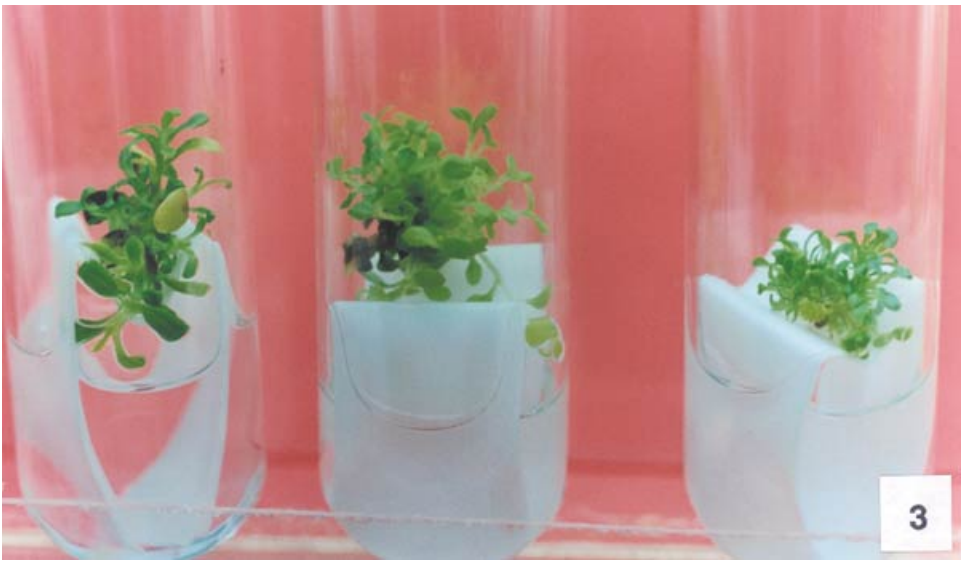
Colored agar sticks are probably an option but would be harder to use compared to powder. Food coloring can be used to add color to your media to indicate what growth regulators it includes.

The initiation media might be one color or clear and might not have any added plant growth regulators. Yellow or Blue might be used for your multiplication media and could include more Cytokinin for shoot formation. Red might indicate more Auxin for rooting.

The agar is usually heated or boiled to dissolve and mix it into your media before it is sterilized. Sometimes it is added to the individual jars of media before being sterilized instead of adding it to the media before filling the containers. Most likely, if added directly to the jars, they will need to be swirled to mix it in before the media solidifies. If you add it to the media and heat it up to get it incorporated first, you should be able to just let the jars of media cool in the pressure cooker to solidify.

Agar is an ideal solidifying agent for microbiological media because of its melting properties and because it has no nutritive value for the vast majority of bacteria but other substitutes might work if you can't get agar.

Liquid media can be used for some tissue culture plants or some sort of support might be needed to keep the culture exposed to the media but not submerged. Cotton, filter paper, perlite, or even sand, coco coir, or peat moss might work. These will make spotting contamination harder (or slower) than a clear agar media but might be a cheaper and more readily available option.



Guar gum is harder to work with but could be tried. Corn starch, potato starch, arrowroot powder, xanthan gum, or other things might also be experimented with. None are likely to have the same properties as agar and they probably require different methods to prepare them.

Plant Growth Regulators or Hormones

Plant growth regulators are simple chemicals that regulate plant growth. Plants produce their own natural regulators known as phytohormones but there are also synthetic growth regulators that can be added to the media to promote specific types of growth. The two most common plant growth regulators are auxin and cytokinin.

Natural plant auxins promote stem elongation, inhibit growth of lateral buds (maintains apical dominance), and facilitate root differentiation. Auxins are naturally produced in the tips of the growing stems and roots (apical meristem) of a plant. This is why if the apical meristem is removed by removing the end of the stem, the lateral buds will be released from apical dominance, and greater branching results.

Auxins induce cell division, cell elongation, and formation of callus in cultures. Auxins are also used as weed killers, rooting powders.

Natural Auxins include Indole butyric acid (IBA), Indole-3-acetic acid (IAA). Synthetic Auxins include 2,4-dichlorophenoxyacetic (2,4-D) and Naphthalene acetic acid (NAA).

The locally available sources of these auxins are not pure so you will need to work out how much is needed to make your solutions to get 1 milliliter equal to 1 milligram of auxin or 1 milliliter equals 0.1 milligram so you can easily add a specific amount to your media.

In the case of the rooting compound, the amount of the active ingredient (Indole-3-butyric acid or IBA) is only 0.10% so you are going to need 10 grams of the rooting compound powder to equal 1 gram of the IBA you need as an auxin.

Assuming you want your solution to contain 1 milligram per milliliter, and you want to make 100 ml of the stock solution, then you need 100 milligrams of the active IBA or 1 gram of the rooting compound.

To dissolve the IBA, you can use a 0.1 Molar solution of Sodium hydroxide (NaOH) or Potassium hydroxide (KOH) to dissolve the chemical. There is a good chance the 90% inactive ingredients in the rooting compound will not dissolve depending on what they are. After mixing the powder in the solvent for a while, you can let it settle and then remove as much of the clear solvent as possible or you can leave it in and not worry about it. Just realize that the undissolved material probably isn't the active ingredient you want so try not to add it to your media but it probably wouldn't do any harm if you did.

You need 4.2 grams of Sodium hydroxide in 1 liter of water to make a 1 Molar solution. 0.42 grams if you only want 100 milliliters.

For Potassium hydroxide, you need 5.6 grams per liter or 0.56 grams per 100 milliliters.

Potassium hydrogen phthalate is considered a stable, reliable chemical for calibrating other acids and bases. It has a molar mass of 204.2 (check my result yourself, please) and has an equivalent weight equal to its molar mass. That means there will be one reactable hydrogen ion in every molecule. Sodium hydroxide, whose molar mass is about 40, also has an equivalent weight equal to its molar mass. It has one reactable hydroxyl ion per molecule.

The equipment I'd use if I were doing this would be some volumetric flasks, a pipette, and a burette. Lets take the simplest example, and I will leave to you to figure out what will work for you. Weigh exactly 0.1 equivalent of KHP (20.42 grams) and carefully put it into a 1 liter volumetric flask. Fill the flask exactly to the neck mark with distilled water. Now you have a solution of 0.1N KHP. This is your calibration solution. Now prepare your NaOH solution the same way--about 4 g/liter in another volumetric flask. Careful, NaOH will dissolve lots of stuff you don't want to dissolve, such as your

eyes and skin and anything wool. You need to know what you're doing in this step. Now, you have a flask of exactly 0.1N acid (the KHP) and approximately 0.1N NaOH. Even if you weigh it precisely, the NaOH picks up so much stuff from the atmosphere that your solutions always need to be calibrated.

Still with me? Now you need a pipette. Put a precise amount of your KHP solution in a flask. Put some of your NaOH solution into a burette. Here you need to know how to titrate. It isn't rocket science, but it does require good technique. Practice! You will need to be pretty good when you get to acetic acid.

You need an indicator--something that changes color when the pH changes. The pH change for these two materials is very fast, and at the "end point" one drop will make the difference in color. Proceed with appropriate caution. So, if you put 10 ml of KHP in your flask, you should expect to see the end point when ABOUT 10 ml of NaOH has been added. If you add precisely 10 ml, your NaOH solution is 0.1N. If you add 11 ml, the solution is obviously weaker than 0.1 N, and would be in fact 0.1 x (10/11) N. Not to belabor the obvious, but your KHP solution has 0.1 equivalents/liter. So, the equivalents in your flask will be 0.1 x ml added/1000. The equivalents of NaOH added will be equal at the end point, so you will know how many equivalents of NaOH you added from your burette. Divide that by the volume, and you have the normality of the NaOH. That is defined as equivalents/litre. Most of work with milliequivalents/millilitre, which is numerically the same.

You won't hit 0.100N. That's fine. You may go through life without ever hitting exactly 0.1000 N NaOH. All you need to know is exactly what it is, and you can calculate everything else.

OK, that last part was just added in as extra information. The exact concentration isn't needed as long as it isn't too strong (which might effect the pH of your media) and if it can dissolve the chemical in it.

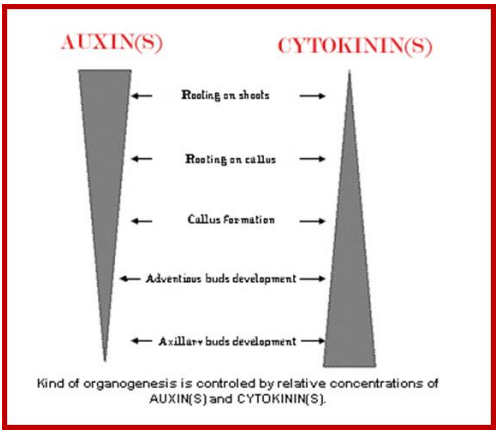
You can use ammonium hydroxide or at least 75% alcohol to dissolve IBA in place of KOH or NaOH. This means that you can probably use household ammonia or 91% rubbing alcohol to dissolve the IBA.

The 2,4-dichlorophenoxyacetic (2,4-D) I got is a little tricky to figure out. The label states it is equivalent to 9.83% of 2,4-dichlorophenoxyacetic acid. Again, that should mean that 1 gram only contains 0.0983 grams (close to 100 milligrams) of the desired 2,4-D. If you assume 1 milliliter weighs 1 gram, then you only need 1 milliliter of the weed killer to make 100 milliliters of a 1 mg/ml stock

Benzyladenine (6-benzylaminopurine, N6-benzylaminopurine, BA or BAP) can be dissolved in 0.1-M hydrochloric acid or 95 or 70% ethanol. It is best to dissolve it and other cytokinins in 70% ethanol because the alcohol is also a sterilant. Solutions of BA and other cytokinins should be stored in a freezer between uses. Prolonged storage of solutions is not advisable.

The 0.1-M Hydrochloric acid can be made using 8.6 milliliters of muriatic acid and enough water to make 1 liter. Start with about half the water and carefully add the acid. Then add enough water to bring it to a full liter. Scaling that down, 0.86 milliliters to 100 milliliters water.

It should also dissolve in 0.1 Molar Sodium or Potassium hydroxide and possibly even ammonium hydroxide but I could be wrong on the last one. Makes me wonder if the IBA or other plant growth regulators can be dissolved in any of the solutions but I haven't done any tests to find out.



100 mL
REORDER NO. 6250
STERILE
USP Sterile Water
Indications: For moistening of wound dressings, wound debridement, and device irrigation. Caution: Not for injection. No antimicrobial or other substance added. Federal (USA) Law restricts this device to sale by or on the order of a physician. Contents sterile unless container is opened or damaged.
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250 mL
REORDER NO. 6270
STERILE
USP Normal Saline
0.9% Sodium Chloride
Indications: For moistening of wound dressings, wound debridement, and device irrigation. Caution: Not for injection. No antimicrobial or other substance added. Federal (USA) Law restricts this device to sale by or on the order of a physician. Contents sterile unless container is opened or damaged.
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Nathon City, Texas USA 76117
www.nurseassist.com
800-649-6800
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Distilled Water
100ml
Store at room temp
Distilled Water
500mL
Store at room temp
Distilled Water
1L
Store at room temp
Distilled Water
1Gallon
Store at room temp
GBiosciences
DO NOT REMOVE PLASTIC CONTAINER FROM CAUTION
THIS SIDE UP FOR STORAGE
TO OPEN, GRASP CAP & PULL UP
GBiosciences.com

Sterile Water

Sterile water is used in tissue culture to rinse off the stronger sanitizing solutions. It is important that the rinse water be sterile to keep from reintroducing contaminants to the items you just cleaned.

Normal 0.9% saline solution contains 9 grams of sodium chloride per liter. Saline solution is usually called normal saline, but it's sometimes referred to as physiological or isotonic saline. It is called normal because its salt concentration is similar to tears, blood and other body fluids (0.9% saline). I have not tested it or found any information if this is safe to use for rinsing plants.

Sterile water can be bought but it is more likely you will make it yourself. Distilled water from the store is not necessarily sterile so you might want to sterilize it in the pressure cooker before using it. It's up to you if you want to chance using it without sterilizing it yourself.

Bottled water or even tap water can be used to make your sterile water instead of distilled water for most uses. Simply boiling the water might be good enough but using a pressure cooker or autoclave is better.

A weak chlorine solution or the addition of other chemicals such as Plant Preservation Mixture (PPM®), Chlorothalonil, Signum fungicide, or other chemicals might be a useful addition to the sterile rinse water or even to your media to help protect the plants from contamination but mentioning this idea often gets all kinds of negative comments usually concerning the dangers of the chemicals.

Be sure to check the available chlorine content of your bleach.

CONCENTRATED FORMULA
For Standard & HE-Machines
ACTIVE INGREDIENT:
Sodium Hypochlorite.....7.5%
OTHER INGREDIENTS:.....92.5%
TOTAL:.....100.0%
(Yields 7.1% available chlorine)
KEEP OUT OF REACH OF CHILDREN
DANGER: CORROSIVE.
FIRST AID: IF IN EYES: Hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. IF ON SKIN OR CLOTHING: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15–20 minutes. IN EITHER CASE, CALL A POISON CONTROL CENTER OR DOCTOR IMMEDIATELY FOR

Notice the available chlorine of this bottle of bleach is 7.1%

7.1% is the same as 71,000 ppm.

A 10% dilution is 7,100 ppm which is still pretty high.

A 1% solution (1 ml bleach to 99 ml water) is still 710 ppm.

A 2% solution (2 ml bleach to 98 ml water) is 1420 ppm.

Even ½ milliliter in 100 ml should be enough (355 ppm) to make a sterile rinse solution.

Approximately 300 ppm solution of available chlorine can possibly be used as a sterile rinse water. This might be easier to make, especially in the field, than sterilizing the water in a pressure cooker or autoclave.

5.25% available chlorine bleach

1 gallon final volume - 1 TBS bleach ~200 ppm solution

1 liter final volume - 1 tsp bleach ~ 263 ppm solution

7.86% available chlorine bleach

1 gallon final volume - 2 tsp bleach ~ 200 ppm solution

1 liter final volume - 1 tsp bleach ~393 ppm solution

2.5 ml bleach ~200 ppm solution

56% available chlorine NaDCC

1 gallon final volume - 1.352 grams ~200 ppm solution

2 grams ~296 ppm solution

1 liter final volume - 0.357 grams ~200 ppm solution

0.5 grams ~280 ppm solution

If you want to be exact, the NaDCC would be 2.028 grams per gallon or 0.636 grams per liter to get 300 ppm (0.03%).

For the use of Chlorothalonil, I'm basing this from a paper called *Chlorothalonil: an effective bacteriostatic agent for bud induction of Acacia auriculiformis under open condition (non-axenic)*

I found this chemical as Ortho Scotts MAX Garden Disease Control Concentrate.

It says the active ingredient is Chlorothalonil 29.6%

If I understand right, this should mean that 1 gram of this stuff has 0.296 grams of the Chlorothalonil.

The paper referenced above says
"The results showed that the suitable type and concentration of bacteriostatic agent was 0.2 g per liter Chlorothalonil."
If I want to make a solution that gives me 0.2 grams per milliliter, I'm figuring 0.2 divided by 0.296 gives me approximately 0.676

So, if I add 6.76 grams of the concentrate to enough water to give me a total of 10 ml, then 1 ml of this solution should give me 0.2 grams of the active chemical. Alternately, just using about 0.7 ml of the full strength liquid per liter should be about the same thing.

I have been testing this chemical as a PPM substitute to help protect the media from contamination.

I made the media on a hot plate stirrer and boiled it to get the agar to melt and mix in. Then I used it in clean but not necessarily sterile containers and didn't bother pressure cooking it at all. I also did this in the open and didn't even use a still air box.

So far all of my tests have stayed clean except for the one that I purposely put mold into. That one got completely covered with the mold so this chemical didn't kill large quantities of mold or stop it from growing. This is why I hesitated sharing this but it seems to be helping and is way cheaper than PPM. It also kind of confirms my suspicion that the PPM in the media was a big part of the reason a microwave could be used instead of pressure cooking the media. I've tried microwave sterilizing media before without using PPM and had mixed results.

Deficiency Chart of Micronutrients
Boron: Discoloration of leaf buds. Breaking and dropping of buds
Calcium: Plant dark green. Tender leaves pale. Drying starts from the tips. Eventually leaf bunds die.
Sulphur: Leaves light green. Veins pale green. No spots.
Iron: Leaves pale. No spots. Major veins green.
Manganese: Leaves pale in color. Veins and venules dark green and reticulated
Copper: Pale pink between the veins. Wilt and drop.
Zinc: Leaves pale, narrow and short Veins dark green. Dark spots on leaves and edges.
Molybdenum: Leaves light green/ lemon yellow/ornge. Spots on whole leaf except veins. Sticky secretions from under the leaf.
Magnesium: Paleness from leaf edges. No spots Edges have cup shaped folds. Leaves die and drop in extreme deficiency.
Potassium: Small spots on the tips, edges of pale leaves. Spots turn rusty. Folds at tips.
Phosphorus: Plant short and dark green. In extreme deficiencies turn brown or black. Bronze colour under the leaf.
Nitrogen: Stunted growth. Extremely pale color. Upright leaves with light green/yellowish.Appear burnt in extreme deficiency.
THE COLOUR REPRESENTED ARE INDICATIVE.
THEY MAY VARY FROM PLANT TO PLANT

The Pressure Cooker

For sterilizing your media, tools, jars, test tubes, and other items needed in this hobby, a normal pressure cooker is commonly used in place of a professional autoclave. A pressure canner or an Instant Pot type pressure cooker can also be used.

The basic idea is that as the pressure increases, the boiling point also increases meaning the temperature can be higher than the normal boiling point of water.

Lb / In2 (P.S.I)	Kg / Cm2	Temperature
5	0.35	107°C (224.6°F)
7	0.47	110°C (230°F)
10	0.70	115°C (239°F)
15	1.05	121°C (249.8°F)
20	1.40	126°C (258.8°F)

Most pressure cookers and pressure canners can operate at 15 PSI but some may be less. Many don't include a pressure gauge and only have a weight that covers a vent pipe to vent at a certain pressure. You will adjust your temperature so it vents or jiggles about 1-4 times per minute. If it is constantly venting, the pressure and therefore temperature is probably a bit too high. More importantly, the steam coming out is water being lost so you might boil it dry and damage the cooker. Mostly though, it is just a waste of energy.

The time required depends on what pressure you are at, what is being sterilized, and the amount of liquid in each jar or container. Normally, 15 to 30 minutes at 15 PSI will be long enough

Volume per container	Time
75-200 ml	20 minutes
200-500 ml	25 minutes
500-1000 ml	30 minutes
1000-1500 ml	35 minutes
1500-2000 ml	40 minutes

Start timing when the pressure reaches the set point (when the weight starts jiggling) and let the pressure drop on its own when you finish. If you are not sterilizing any liquids, you could technically open the vent after sterilizing so you can open it up faster but liquids will boil if the pressure is released too fast. This can boil over making a mess and losing your sterile media or other liquid. It can also blow off the lids or damage the container.

When you intend to pour your agar containing media after it is sterilized, you will need to catch it before it solidifies. About 55-75°C (131-167°F) is the right range for pouring. You might will still want heat resistant gloves or some protection for your hands at this temperature. If you pour at too high of a temperature, you could damage your container (jars or petri dishes) but mostly the problem is you will get a lot of condensation as well as the added danger from the heat.

If you wait too long, the media can start to solidify before you get it all poured. You can remelt the media carefully in the pressure cooker, a hot water bath, or a microwave. Make sure the lid is loose and don't use a metal lid if using the microwave. Heat about one minute at a time on low power until all of the agar is melted. Swirl the bottle in between heating it to make sure the agar is melting evenly. Watch that the media doesn't boil over while it is heating.

The pressure cooker can also be used to sterilize your tools, a small plate for doing your cutting, or many other things. Glass, metal, or autoclavable plastics like Polypropylene (recycle code 5) and polypropylene copolymer (PPC is a bit softer but has better impact strength, is tougher and more durable than homopolymer polypropylene), and Fluoropolymer (Teflon) containers are all considered safe to be autoclaved repeatedly. Other plastics such as HDPE, LDPE, PET, and PETG cannot be autoclaved and should instead be sterilized using gas.

You can wrap things like Q-tips, tooth picks, scalpels and blades, and many other things in aluminum foil before sterilizing them to keep them sterile until they are opened for use. A bunch of foil packets can be put into a canning jar or other container as another barrier to contamination.

From teach.genetics.utah.edu website, I found this information.which might be helpful.

Pre-sterilized items are great time savers. The following items come sterile in the package:

Toothpicks in unopened boxes are sterile and can be used directly from the box. Use scissors to cut a small hole (about 1/4 in. across) in one corner of the box to serve as a dispenser. Cover the hole with tape when not in use.

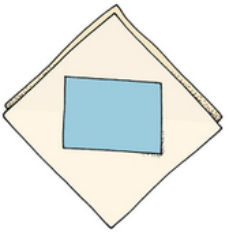
You can also use individually wrapped toothpicks.

Facial tissues: Discard the top one or two tissues that have been exposed to air and dust. The ones underneath will be sterile.

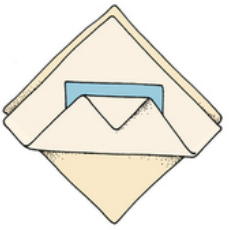
Paper towels (the kind in rolls): The side that contacts the roll is sterile.

Items specifically made and packaged for microbiology, including Petri dishes, disposable inoculating loops and tubes, and individually wrapped transfer pipets.

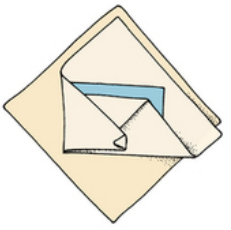
You will have to decide if you want to trust that these items are sterile. Plastic bags are also supposed to be sterile right out of the package.



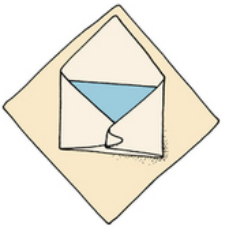
1. Place two wrappers on flat surface with one point toward you. Place item to be wrapped in center of wrapper with its length parallel to you.



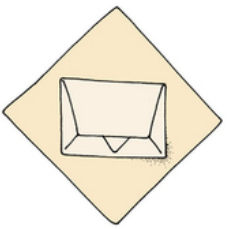
2. Fold corner nearest you over item until it is completely covered. Fold corner back toward you 2 to 3 inches.



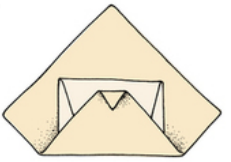
3. Fold left side of wrapper over and parallel to item. Fold end of corner back 2 to 3 inches.



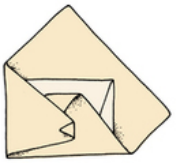
4. Repeat with right side. Lap center folds at least 1/2 inch.



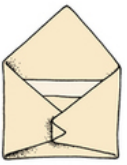
5. Tuck in side edges of remaining corner to eliminate any direct opening to item. Bring top corner down to bottom edges and tuck in, leaving point for opening.



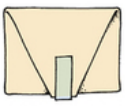
6. Repeat step 2.



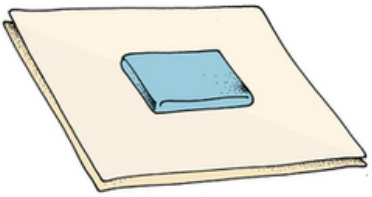
7. Repeat step 3.



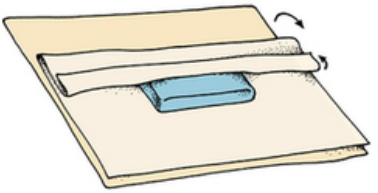
8. Repeat step 4.



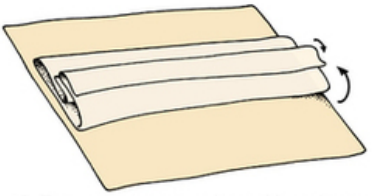
9. Bring point of wrapper completely around package and seal with appropriate tape.



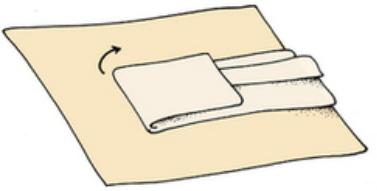
1. Place items assembled for pack in center of two sheets of wrapping material.



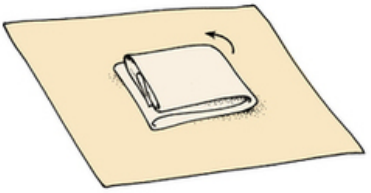
2. Fan-fold open end away from you over items. Cuff top layer.



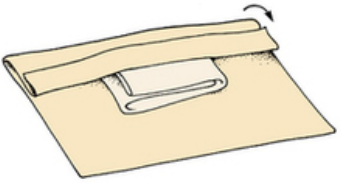
3. Repeat same procedure with end toward you, lining up cuff directly on top of first cuff.



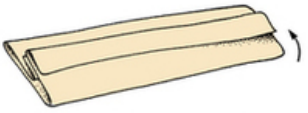
4. Miter left end and fold neatly up and over top of pack.



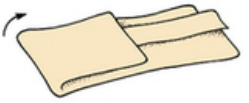
5. Repeat with right side of pack.



6. Repeat step 2.



7. Repeat step 3.



8. Repeat step 4.

9. Repeat step 5 and securely affix with pressure-sensitive indicator tape over end.

Vitamin B1 (Thiamin/Thiamine)

There is some debate on whether this vitamin helps with seed germination but some studies show a significant increase in the rate of germination when treated with this vitamin.

The solubility of thiamine is pretty high and 1 gram can dissolve in 1 ml of water. Soaking the seeds in a 3 mM solution for 24 hours at 25° C (77° F) was the strength and time I found recommended for bean seeds. Lower temperatures (5° C or 41° F) showed no response to the treatment.

This chemical can be easily purchased as vitamin B1 pills. The bottle will state the amount of vitamin contained in each pill but they will also contain other fillers. Crushing up the pills and soaking them in water should extract the vitamin and any undissolved sediment is likely just fillers.

To get the 3 mM solution, you need approximately 796 mg per liter or 80 mg for 100 ml. Some vitamin B1 comes as 100 mg pills but can be 250 or 500 mg per tablet in some.

Assuming each pill contains 100 mg of the vitamin B1, one pill dissolved in 12.5 ml water should make a 3 mM solution. Two of the 100 mg pills should make 25 ml or four pills to get 50 ml.

For 250 mg pills, each pill should make 31.4 ml of the 3 mM solution.

If you start with 500 mg pills, each pill should make 62.8 ml of the 3 mM solution.

Thiamine decomposes at 248° C (478.4° F) which is higher than the temperature of a pressure cooker or autoclave so I assume it can be sterilized in your media or soaking liquid.

LIQUINOX
Start
A ROOT STIMULANT
0-2-0
Vitamin
B-1
PLUS CHELATED IRON and PHOSPHATE WITH YUCCA EXTRACT BASE
TRANSPLANT SOLUTION
FOR ROOT GROWTH
REDUCES TRANSPLANT SHOCK
NET 1 GAL. (128 FL.OZ.) 3.785 L

REDUCES Transplant Shock
Lilly Miller
ULTRAGREEN
Vitamin B1
Plant Starter
Provides Chelated Iron, Manganese and Zinc
For Flowers, Vegetables, Seeds, Trees and Shrubs
NET CONTENTS/CONTENIDO NETO 1 GAL. (3.7 l)

PLATINUM SERIES
PERFORMANCE GUARANTEE
CYCO
B1 Boost
Renfort B1

Examples of Kit Contents

There are several companies selling tissue culture kits for home or school use. The contents and prices vary depending on what is included and what you will need to provide yourself. Some will have tubes, jars, petri dishes, or other containers or they might include special plastic lids that fit baby food jars which you need to provide yourself.

Most will provide enough media to make at least one liter. Some have the plant growth regulators already mixed into the media or you might get a selection of pre-mixed or at least pre-weighed growth regulators you can add to the media as you desire.

Most will provide the agar or gellan gum to turn the media into a soft gel.

Forceps and a scalpel or knife to hold, cut, and transfer the cultures are usually included.

You will probably also get some pipettes, a small syringe, an assortment of measuring spoons, and possibly other measuring cups or beakers.

Some pH papers or strips and possibly pH adjusting chemicals. Many people will just use vinegar and baking soda to adjust the pH.

Here I will try to list the items I feel would make a good, complete tissue culture kit. You won't find this as a kit so you will have to pick and choose what you want in your own kit and either buy them online, find them locally, make them yourself, or recycle and repurpose items to use. Some will be put together and sterilized as you need them.

Tools:

Still-air box - see the still air-box chapter

Forceps - 8 inch long is a good size but an assortment could be useful. Used for all kinds of moving and holding jobs.

Scalpel, knife, scissors, and other cutting tools - A dissection kit can be bought which includes these tools. Other garden cutters , kitchen paring knives, wire cutters, etc. might be useful.

Inoculating loop - This can be bought or homemade. Nichrome wire is ideal for making this because it can be repeatedly heated without damage but even bread tie wire can be used to make temporary loops. Not overly useful for plant tissue culture but can be useful if you are working with mushroom spores, fern spores, tiny seeds, yeast cultures, bacteria cultures (tempeh), or for transferring tiny amounts of liquids like dish soap, surfactants, etc.

Inoculating needle - This is similar to the inoculating loop often made of nichrome wire. Often a blunt point. Not a lot of uses for plant tissue culture but could be useful if working under magnification.

Sterile toothpicks - Sterilized wrapped in foil or in a tube or jar and pressure cooked. Too short for working in jars or tubes but good for working in a petri dish. Cheap and disposable.

Sterile Q-tips - Sterilized the same as toothpicks. Cheap and disposable.

Paint Brush - A small paint brush can be useful for washing/brushing some plant pieces before surface sterilizing them. Tough to clean outdoor cuttings can be difficult to get cleaned up so might need a little extra brushing in the initial cleaning steps.

Spray Bottle - This can be filled with 70% isopropyl alcohol for cleaning surfaces, outsides of containers, hands, etc. You might find a sprayer lid that will fit right on a rubbing alcohol bottle. You can sometimes find alcohol or hydrogen peroxide sold in a spray bottle. A bleach solution can also be put in a spray bottle for cleaning stuff. You will probably want at least a couple spray bottles but make sure you label them good so you don't get mixed up.

Dropper Bottles - Glass dropper bottles can be bought new or you can recycle old eye drop bottles or other bottles to hold various liquids. Dish soap or another surfactant would be good to have in a small bottle like this. A bigger contact lens solution bottle for cleaning solutions like the spray bottles.

Jars and Bottles - You can find uses for many jars and bottles. Besides the obvious baby food jars, some other foods and drinks come in glass bottles that could be repurposed as tissue culture containers or for many other uses.

Tall Shotglass - This, or something else tall and narrow to soak forceps and scalpel/ knife in to clean them between transfers. Fill it with alcohol or a bleach solution. Bleach will rust your tools but will work. The tall and narrow container will let the sanitizing solution contact more of the tools without using a lot of solution. It might need supported in a jar or something else to keep it from falling over.

Cutting Surface - This should be an easily cleaned surface to put the plant pieces or cultures to be cut, divided, or trimmed before putting it into the media. A small plate or a glass petri dish can work but a ceramic tile or piece of glass will also work. You can clean it off with alcohol or bleach solution. You could also use sterilized pieces of paper towel on top with some alcohol or bleach to do the cutting on. This can then be thrown away along with the trimmed pieces.

Alcohol Lamp/Bunsen Burner - Many hobbyists don't use any flames because of the fire hazard it poses but this can be used to flame sterilize metal inoculating loops and needles. Scalpel blades can also be sterilized with a flame but it will dull the blade, change its color, and cause it to rust and flake off. It can also be used to heat or boil small amounts of water, media, or other liquids. Flaming the opening of a test tube, flask, or bottle can help kill spores and other contaminants that may be on them but another purpose is to heat the air inside causing it to flow out instead of in and lessening the chance of contamination entering. It is flamed quickly and not really heated much so some people might flame plastic tubes but that could cause burning, melting, or distorting them. A propane or butane torch makes a good substitute for the alcohol lamp or Bunsen burner. They don't have the same old time laboratory feel to them but are convenient and often have a push button igniter.

Beakers and Flasks - These are useful for heating liquids on a hot plate or with the alcohol lamp or Bunsen burner or in a microwave. They handle thermal shock better than a jar and they have a flat bottom which works good with a magnetic stir bar and stir plate. The flask is often used to grow yeast starter cultures and can be used as tissue culture vessels although they are rather expensive compared to other options. They often have volume measurement markings on them so can be used to measure liquids but are not as accurate as a graduated cylinder.

Graduated Cylinder - These may not be needed but are more accurate for measuring liquids. They come in different sizes depending on the volume you need to measure and can be made from glass or plastic.

Measuring Spoons/Cups - These can be used for measuring powders and liquids where absolute precision is not needed. In addition to the normal measuring spoons like you would use for cooking, you can get special miniature spoons that measures in Tad (¼ teaspoon), Dash (⅛ teaspoon), Pinch (1/16 teaspoon), Smidgen (1/32 teaspoon), and Drop (1/64 teaspoon). Measuring cups can also be

used for larger amounts. A glass measuring cup can be a good way to measure liquids if you don't need absolute accuracy.

Digital Scale - You need a scale to weigh out the various chemicals you will be using. The ability to accurately weigh things down to the milligram level would be great but you are not likely to find a scale that accurate at an affordable price. Cheap digital scales capable of weighing 0.01 grams are available and should be good enough. A kitchen scale can weigh larger amounts but is not as accurate as one with a maximum capacity of 100 grams or 500 grams.

Hot Plate/Magnetic Stirrer - This is often found as a single unit and can be useful for stirring liquids while they are heated to help dissolve your media or chemicals. The stir plate can also be found as a separate tool and works good when cleaning plant cuttings. The magnetic stir plate can be homemade often using a computer fan or small motor to rotate a strong magnet. A speed control is used to slow and regulate the rotating speed down to keep the stir bar from being thrown and to keep from damaging the plant pieces.

Magnetic Stir Bar - This is used with a magnetic stir plate. Most often it is a teflon coated magnet so it can handle heat and is resistant to most chemicals. They are easily lost and kind of expensive so you might use a piece of a nail, a screw, or even a bent paper clip if you are not mixing acids or strong bases. These substitutes might scratch your glassware though. Along with the stir bar, it is nice to have a stir bar retriever. Another strong magnet can be used to stick the stir bar to the glass when pouring the liquids out or it can drag the stir bar up the side and out for removal.

Orbital Shaker/Tube Rotator/Rotary Shaker/Laboratory Rocker - These are probably outside the budget of a home tissue culture hobbyist but you might find an imported or used one at a lower price. Depending on the type you find, these can often hold multiple tubes, jars, bottles, etc. and agitate them all at the same time. They don't need a magnetic stir bar in each vessel and they cause less foaming when surface sterilizing items with dish soap or some other surfactant. They are probably not needed but something to consider if you find one cheap or even free. A broken one might be easily repaired if you are mechanically inclined.

pH meter or pH papers - You will need a way to test the pH of your media so you can adjust it to the desired range. Cheap pH meters are available online but may not be accurate or durable. They need calibration to ensure they are set right. If you do use a meter, check the media BEFORE adding the agar. The agar will gel on the meter's electrode and will be hard to clean and might damage it.

You can find pH papers online. Universal indicator papers can read between 1 and 14. Most tissue culture media should have a pH of 5.2-5.8 so you might want a narrow range pH paper in that range to get closer.

The pH of a solution is a measure of the concentration of hydrogen ions in the solution. The pH scale extends from very acid (0) to very alkaline (14) with 7 being the "neutral" point. The pH of most tissue culture media is adjusted to around 5.7 ± 0.1 before autoclaving. It pH will usually drop slightly during autoclaving but it is not normally readjusted again. The pH can influence the solubility of ions in nutrient media, the ability of agar to gel, and the subsequent growth of cells. For example, if the pH is lower than about 4.5, agar medium will not gel.

Culture Vessels - You need something to grow your cultures in. This can be baby food jars with the original, metal lids or special plastic caps. Test tubes, centrifuge tubes, jars, bottles, flasks, etc. might be options. Petri dishes (disposable plastic, reusable plastic, or glass) can be used for some things. Mushroom cultures, yeast cultures, and bacteria cultures can be started, cleaned up, isolated, and grown in petri dishes. Tiny seeds, spores, or plant pieces can also be started using petri dishes in place of jars or other vessels.

Brushes for Cleaning - An assortment of bottle and tube brushes will be helpful to clean things for reuse. These can be bought online or you might be able to find something usable locally. You can even make something yourself. Wire, coat hangers, dowel rods, etc. combined with pieces of a sponge, rags, or scrub pads can be used to help clean the insides of the vessels. Maybe even your long tweezers or forceps and a paper towel.

Chemicals:

You will use many chemicals or ingredients while doing plant tissue culture. Many can be normal household chemicals and others are harder to find and will need to be ordered. Most of them have at least some dangers so you should do your own research if you plan to keep and use them. They should be labeled and stored out of reach of children and others who may need protection from their own curiosity. Some chemicals will have specific storage requirements like being kept cold or kept away from certain other chemicals. Again, do your own research and use common sense.

Dihydrogen monoxide (DHMO) - You will need a lot of this chemical but, like most things, it can be dangerous so you need to do your research and decide if the dangers are worth the risk of having it around and using it in your work. Some of the properties found on Wikipedia for this chemical are:

Dihydrogen monoxide:

is also known as hydroxyl acid, and is the major component of acid rain.
contributes to the "greenhouse effect".
may cause severe burns.
contributes to the erosion of our natural landscape.
accelerates corrosion and rusting of many metals.
may cause electrical failures and decreased effectiveness of automobile brakes.
has been found in excised tumors of terminal cancer patients.
Despite the danger, dihydrogen monoxide is often used:

as an industrial solvent and coolant.
in nuclear power plants.
in the production of styrofoam.
as a fire retardant.
in many forms of cruel animal research.
in the distribution of pesticides. Even after washing, produce remains contaminated by this chemical.
as an additive in certain "junk-foods" and other food products.

Water (H2O) - If you hadn't figured it out, this is the same chemical as Dihydrogen monoxide. Regular tap water will be used to wash all your tools, jars, etc. It can be used for actually making your media and other solutions but many people choose to use distilled water. Even if you use distilled water and especially if you use tap water, you will want some that has been sterilized. This is usually done in a pressure cooker and you could make several smaller jars or bottles and save it for later use. It is probably better to make multiple small containers instead of one large amount because it might not be completely sterile once you open it. Smaller volumes take less time to sterilize too and you can often fit a small jar or bottle of water in with other things you are sterilizing.

Pure water at 4°C (39.2°F) weighs 1 gram per milliliter. At 21°C (69.8°F), it weighs about 0.998 grams. At 25°C (77°F), it weighs about 0.997 grams. At 37°C (98.6°F), it weighs slightly over 0.993 grams. Therefore at a normal room temperature, figuring 1 gram per milliliter, you would be off by only 2 or 3 milliliters.

A teaspoon of water is almost 5 milliliters, a tablespoon is close to 15 milliliters, and a cup is 236.59 milliliters. You won't be exact measuring with this way but it can be a fast way to fill your jars or tubes with media.

Volume	Weight (Oz)	Weight (Lb)	Weight (G)	Weight (Kg)
1 teaspoon	0.1739 oz	0.0109 lb	4.929 g	0.004929 kg
1 tablespoon	0.5216 oz	0.0326 lb	14.787 g	0.0148 kg
1 cup	8.345 oz	0.5216 lb	236.59 g	0.2366 kg
1 pint	16.691 oz	1.043 lb	473.18 g	0.4732 kg
1 quart	33.382 oz	2.086 lb	946.35 g	0.9464 kg
1 gallon	133.53 oz	8.345 lb	3,785.4 g	3.785 kg
1 milliliter	0.0353 oz	0.002205 lb	1 g	0.001 kg
1 liter	35.274 oz	2.205 lb	1,000 g	1 kg

Sterile Water - Sterile water is used for rinsing off other disinfecting chemicals from seeds and plants before putting them into the media. It can be made by pressure cooking tap water, filtered water, or distilled water. A sterile rinse water can also be made from around 0.03% (300 ppm) bleach or NaDCC solution. If your bleach is 7.86% available chlorine, 1 ml of bleach mixed up to a final volume of 250 ml should give you a 315 ppm solution. If your bleach is only 5.25% available chlorine, you need 1.5 ml of bleach to make 250 ml of solution. If you want to make a cup of this sterile rinse water instead of 250 ml, your solution would be 334 ppm if you still used milliliters to measure the bleach. If you used ¼ teaspoon of bleach to make a cup of solution, you would end up at 274 ppm if using the 5.25% available chlorine bleach or about 410 ppm if using the stronger 7.86% available chlorine bleach.

The sterile water, either sterilized in a pressure cooker or chemically sterilized water, would be good to include in your portable culture collecting kit or kept on hand in your lab. If you keep a small bottle of bleach in your kit, you can refill the bottle with tap water and add the required amount of bleach to sterilize it. Waiting about a half an hour after you mix it up for the

chlorine to mix in and kill any contamination would be a good idea. This would be especially true if you are going to use questionable water sources and you might want to wait longer to be sure if you have the time.

Bleach - This will have many uses in your tissue culture hobby. Try to get it as unscented if possible. Check the label to find the available chlorine content. This will be needed to calculate the strength of your solutions. To make it easier to work with and take less space, The bleach can be transferred carefully into a smaller bottle. Label the bottle with the name of the contents and the active chlorine content. Depending on how much bleach you intend to make, a dropper bottle of bleach might be enough and would make measuring by the drop convenient.

Baking Soda - This household chemical, Sodium bicarbonate, is often used to raise the pH of your media. If you mix a Smidgen spoon (1/32 teaspoon) of baking soda with about 20 ml of water, it will make around a 0.1 molar solution. This can be used to adjust the pH easier than trying to do it with the dry powder.

Baking soda is also good to neutralize acids so it is good to have around in case of spills or if you get acids like Vinoxide-HTC on you.

Also used along with bleach to make Dakin's solution.

Vinegar - You want distilled white vinegar which is a 5% aqueous solution of acetic acid. This can be used to adjust the pH of your media or used to make Vinoxide HTC.

Hydrogen Peroxide - This is another common chemical you can easily find locally as a 3% solution. It can be used full strength, diluted at various strengths, or used to make Vinoxide HTC.

Alcohol - Rubbing alcohol (isopropyl alcohol) can be found as a 70% or a 91% solution. The 70% is better for disinfecting purposes because it takes longer to evaporate. The 91% can be used as fuel in an alcohol lamp or you can dilute it to make 70%. Divide the higher concentration by the lower concentration and subtract one, to get the amount of water to add to alcohol, by ratio of parts. In this case, $91 \div 70 = 1.3$ then $1.3 - 1 = 0.3$

Mix 0.3 parts of water with 1 part 91% alcohol. Scale it up to be more convenient and you need 3 parts water with 10 parts 91% alcohol to make the 70% solution.

You might find rubbing alcohol sold as a 50% solution. This can be used and it sometimes comes in a nice little spray bottle but 70% would be better as a sanitizing solution.

The 91% is better for removing permanent ink marker from your jars. If all you have is 50% or 70%, take a little bit and add table salt about 1 part salt to 3 parts alcohol. Shake it up and then let it sit undisturbed until it separates into two layers. The top layer is the stronger, dehydrated alcohol and the bottom layer is the salt water. This works for isopropyl alcohol because the solubility of sodium chloride in it is only 30 milligrams per liter. The salt is soluble in ethanol at 650 mg per liter and 14 grams per liter in methanol so this trick won't work unless you are using isopropyl alcohol.

Denatured alcohol is often a mixture of ethanol and methanol. I couldn't quickly find if it is 99 or 100% but about 4 parts denatured alcohol and 10 parts water should get close to a 70% solution. Because it likely contains methanol, don't use this as a hand sanitizer and avoid contact with your skin but it might be an option for wiping down surfaces or tools. It is a good fuel for an alcohol lamp though.

Surfactants - Surfactants (Surface Active Agents) are used to lower the surface tension of the water, bleach, or other cleaning solutions. Just a drop or two is usually enough. Dish soap or baby shampoo are examples that are sometimes used. Automatic dishwasher drying agents are another choice. There are commercial surfactants that are sold for use with insecticides, fungicides, herbicides, and other plant chemicals.

Sugar - Regular table sugar is a soluble carbohydrate called sucrose. Carbohydrates are an important component for plant growth and development *in vitro* where the conditions are not suitable for photosynthesis. Table sugar is cheap and available. It's normally used at a concentration of 2-3% (20-30 grams per liter).

Coconut Water - coconut water can be used at 10-15% (100-150 milliliters per liter) as an additive to tissue culture medium. Coconut water induces division and growth in plant cells when added to auxin-containing media. It is also found to induce growth in callus and suspension cultures and induce morphogenesis in cultured explants. In some species, coconut water alone can induce callus growth. Coconut water has been reported to enhance callus induction, shoot development and multiplication in tissue culture of plants. Nine cytokinins have been identified from coconut water including Zeatin-o-glucocide, Dihydrozeatin-o-glucocide, trans-zeatin-riboside-5-monophosphate, Kinetin (free-base and riboside), Gibberillins (GA1 and GA3), Indole-3-acetic acid, and ABA (Absciscic acid).

Other Organic Additives - You might want to research and experiment with other media additives. Some examples and amounts are:

young coconut water at 70 milliliters per liter
mature coconut water at 50 milliliters per liter
banana and tomato juice at 50 milliliters per liter
papaya juice at 20 milliliters per liter